

CLAIMS

1. A method for inducing a population of CD8<sup>+</sup> T cells to proliferate, comprising:

- a) activating a population of T cells, and  
b) stimulating a CD9 antigen on the surface of the T cells with a ligand which binds the CD9 antigen, the activating and stimulating steps thereby inducing proliferation of the T cells.

2. The method of claim 1, wherein the population of T cells is activated by contacting the T cells with an anti-CD3 antibody.

3. The method of claim 2, wherein anti-CD3 antibody is an anti-human CD3 monoclonal antibody.

4. The method of claim 3, wherein the anti-CD3 antibody is immobilized on a solid phase surface.

5. The method of claim 1, wherein the population of T cells is activated by contacting the T cells with an anti-CD2 antibody.

6. The method of claim 1, wherein the population of T cells is activated by contacting the T cells with a protein kinase C activator and a calcium ionophore.

7. The method of claim 1, wherein the ligand binds a peptide comprising an amino acid sequence

(Xaa<sub>1</sub>)<sub>n</sub>-Gly-Xaa<sub>2</sub>-Trp-Leu-Xaa<sub>3</sub>-Xaa<sub>4</sub>-Asp(Glu)-(Xaa<sub>5</sub>)<sub>n</sub> (SEQ ID NO: 5), wherein Xaa<sub>4</sub> may or may not be present, Xaa<sub>1</sub>, Xaa<sub>2</sub>, Xaa<sub>3</sub>, Xaa<sub>4</sub> and Xaa<sub>5</sub> are any amino acid residue and n = 0-20.

8. The method of claim 7, wherein Xaa<sub>2</sub> is Cys, Ile or Leu, Xaa<sub>3</sub> is Leu or Arg and Xaa<sub>4</sub>, if present, is Arg, Pro or Phe.

9. The method of claim 2, wherein the ligand is a monoclonal antibody ES5.2D8.

10. The method of claim 1, further comprising contacting the T cells with an antigen or portion thereof.

11. The method of claim 1, further comprising  
c) monitoring proliferation of the T cells in response to continuing exposure to the ligand; and  
d) reactivating and restimulating the T cells when the rate of T cell proliferation has decreased to induce further proliferation of the T cells.

12. The method of claim 11, further comprising repeating the steps (c)-(d) to produce a population of T cells increased in number of from about 100- to about 100,000-fold the original T cell population

13. The method of claim 9, further comprising  
c) monitoring proliferation of the T cells in response to continuing exposure to the monoclonal antibody ES5.2D8; and  
d) restimulating the T cells with the anti-CD3 antibody and the monoclonal antibody ES5.2D8 when the rate of T cell proliferation has decreased to induce further proliferation of the T cells.

14. The method of claim 13, further comprising repeating steps (c)-(d) to produce a population of T cells increased in number of from about 100- to about 100,000-fold the original T cell population.

15. A method for stimulating a population of CD8<sup>+</sup> T cell to proliferate, comprising  
a) contacting a population of T cells with  
(1) a first agent which stimulates a TCR/CD3 complex-associated signal in the T cells; and  
(2) a second agent which stimulates a CD9 antigen on the surface of the T cells.

16. The method of claim 15, wherein the first agent is an anti-CD3 antibody.

17. The method of claim 16, wherein anti-CD3 antibody is an anti-human CD3 monoclonal antibody.

18. The method of claim 17, wherein the anti-CD3 antibody is immobilized on a solid phase surface.

19. The method of claim 15, wherein the second agent is a ligand which binds a peptide comprising an amino acid sequence

(Xaa<sub>1</sub>)<sub>n</sub>-Gly-Xaa<sub>2</sub>-Trp-Leu-Xaa<sub>3</sub>-Xaa<sub>4</sub>-Asp(Glu)-(Xaa<sub>5</sub>)<sub>n</sub> (SEQ ID NO: 5),  
wherein Xaa<sub>4</sub> may or may not be present, Xaa<sub>1</sub>, Xaa<sub>2</sub>, Xaa<sub>3</sub>, Xaa<sub>4</sub> and Xaa<sub>5</sub> are any amino  
acid residue and n = 0-20.

20. The method of claim 19, wherein Xaa<sub>2</sub> is Cys, Ile or Leu, Xaa<sub>3</sub> is Leu or Arg  
and Xaa<sub>4</sub>, if present, is Arg, Pro or Phe.

21. The method of claim 16, wherein the second agent is a monoclonal antibody  
ES5.2D8.

22. The method of claim 16, further comprising:

- b) separating the anti-CD3 antibody from the T cells and second agent;
- c) monitoring proliferation of the T cells in response to continuing  
exposure to the second agent; and
- d) restimulating the T cells with the anti-CD3 antibody and the second  
agent when the rate of T cell proliferation has decreased to induce further proliferation of the  
T cells.

23. The method of claim 22, further comprising repeating steps (b)-(d) to produce  
a population of T cells increased in number of from about 100- to about 100,000-fold the  
original T cell population.

24. A method for stimulating a population of CD8<sup>+</sup> T cells to proliferate,  
comprising:

- a) contacting the population of T cells with an anti-CD3 antibody and a  
ligand which binds a CD9 antigen on activated T cells, under conditions appropriate for  
proliferation of the T cells;
- b) separating the anti-CD3 antibody from the T cells and the ligand;
- c) monitoring proliferation of the T cells in response to continuing  
exposure to the ligand; and
- d) restimulating the T cells with the anti-CD3 antibody and the ligand  
when T cell proliferation has decreased to induce further proliferation of the T cells.

25. The method of claim 24, further comprising repeating steps (b)-(d) to produce  
a population of T cells increased in number of from about 100- to about 100,000-fold the  
original T cell population.

26. The method of claim 24, wherein the ligand binds a peptide comprising an amino acid sequence

(Xaa<sub>1</sub>)<sub>n</sub>-Gly-Xaa<sub>2</sub>-Trp-Leu-Xaa<sub>3</sub>-Xaa<sub>4</sub>-Asp(Glu)-(Xaa<sub>5</sub>)<sub>n</sub> (SEQ ID NO: 5),  
5 wherein Xaa<sub>4</sub> may or may not be present, Xaa<sub>1</sub>, Xaa<sub>2</sub>, Xaa<sub>3</sub>, Xaa<sub>4</sub> and Xaa<sub>5</sub> are any amino acid residue and n = 0-20.

27. The method of claim 26, wherein Xaa<sub>2</sub> is Cys, Ile or Leu, Xaa<sub>3</sub> is Leu or Arg and Xaa<sub>4</sub>, if present, is Arg, Pro or Phe.

28. The method of claim 25, wherein the anti-CD3 antibody is OKT3 and the ligand is a monoclonal antibody ES5.2D8.

29. The method of claim 24, wherein the population of CD8<sup>+</sup> T cells is tumor infiltrating lymphocytes obtained from an individual afflicted with cancer and the method further comprises restoring the T cells to the individual.

30. The method of claim 29, further comprising genetically transducing the T cells and restoring the transduced T cells to an individual.

31. A substantially homogeneous CD8<sup>+</sup> T cell population produced by the method of claim 25.

32. A method for stimulating a population of CD8<sup>+</sup> T cells to proliferate, comprising:

a) obtaining peripheral blood leukocytes from an individual;  
b) isolating a population of CD8<sup>+</sup> T cells from the peripheral blood leukocytes by negative selection with a combination of antibodies directed to surface markers unique to the cells negatively selected;

c) contacting the population of CD8<sup>+</sup> T cells with an anti-CD3 antibody immobilized on a solid phase and a ligand which binds a CD9 antigen present on activated T cells, under conditions appropriate for stimulating proliferation of the T cells;

d) separating the anti-CD3 antibody from the T cells and the ligand;

e) monitoring proliferation of the T cells in response to continuing exposure to the ligand by examining cell size; and

f) restimulating the T cells with the anti-CD3 antibody and the ligand when T cell size has decreased to induce further proliferation of the T cells.

33. The method of claim 32, further comprising repeating steps (d)-(f) to produce a population of CD8<sup>+</sup> T cells increased in number of from about 100- to about 100,000-fold the original T cell population.

5 34. The method of claim 32, wherein the ligand binds a peptide comprising an amino acid sequence

(Xaa<sub>1</sub>)<sub>n</sub>-Gly-Xaa<sub>2</sub>-Trp-Leu-Xaa<sub>3</sub>-Xaa<sub>4</sub>-Asp(Glu)-(Xaa<sub>5</sub>)<sub>n</sub> (SEQ ID NO: 5), wherein Xaa<sub>4</sub> may or may not be present, Xaa<sub>1</sub>, Xaa<sub>2</sub>, Xaa<sub>3</sub>, Xaa<sub>4</sub> and Xaa<sub>5</sub> are any amino acid residue and n = 0-20.

10 35. The method of claim 34, wherein Xaa<sub>2</sub> is Cys, Ile or Leu, Xaa<sub>3</sub> is Leu or Arg and Xaa<sub>4</sub>, if present, is Arg, Pro or Phe.

15 36. The method of claim 32, wherein the anti-CD3 antibody is OKT3 and the ligand is a monoclonal antibody ES5.2D8.

20 37. The method of claim 32, wherein the population of CD8<sup>+</sup> T cells is tumor infiltrating lymphocytes obtained from an individual afflicted with cancer and the method further comprises restoring the T cells to the individual.

25 38. A monoclonal antibody which specifically binds a CD9 antigen present on activated T cells.

30 39. The monoclonal antibody of claim 38, which specifically binds a peptide comprising an amino acid sequence

(Xaa<sub>1</sub>)<sub>n</sub>-Gly-Xaa<sub>2</sub>-Trp-Leu-Xaa<sub>3</sub>-Xaa<sub>4</sub>-Asp(Glu)-(Xaa<sub>5</sub>)<sub>n</sub> (SEQ ID NO: 5), wherein Xaa<sub>4</sub> may or may not be present, Xaa<sub>1</sub>, Xaa<sub>2</sub>, Xaa<sub>3</sub>, Xaa<sub>4</sub> and Xaa<sub>5</sub> are any amino acid residue and n = 0-20.

35 40. The monoclonal antibody of claim 39, wherein Xaa<sub>2</sub> is Cys, Ile or Leu, Xaa<sub>3</sub> is Leu or Arg and Xaa<sub>4</sub>, if present, is Arg, Pro or Phe.

41. A hybridoma designated by ATCC Accession No. HB11374.

42. A monoclonal antibody produced by the hybridoma of claim 65.

43. A peptide comprising an amino acid sequence

(Xaa<sub>1</sub>)<sub>n</sub>-Gly-Xaa<sub>2</sub>-Trp-Leu-Xaa<sub>3</sub>-Xaa<sub>4</sub>-Asp(Glu)-(Xaa<sub>5</sub>)<sub>n</sub> (SEQ ID NO: 5),  
wherein Xaa<sub>4</sub> may or may not be present, Xaa<sub>1</sub>, Xaa<sub>2</sub>, Xaa<sub>3</sub>, Xaa<sub>4</sub> and Xaa<sub>5</sub> are any amino  
acid residue and n = 0-20.

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44. The peptide of claim 43, wherein Xaa<sub>2</sub> is Cys, Ile or Leu, Xaa<sub>3</sub> is Leu or Arg  
and Xaa<sub>4</sub>, if present, is Arg, Pro or Phe.

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